

SULPHUR AND NITROGEN CONTENT OF ALFALFA GROWN UNDER VARIOUS CONDITIONS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY

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Introduction

Various investigators show a considerable variation in the analyses of the sulphur content of alfalfa, as well as in the proportion of organic and inorganic sulphur in the crop. It was thought well to analyze alfalfa from a number of selected places to see to what degree these variations could be explained by different sources of the crop, and to what degree by the personal factor of applying the none too reliable fusion methods, which probably always involve more or less volatilization and loss of sulphur. The very extensive Oregon work (11) has shown that the acre yield of alfalfa is enormously increased on their plats by the application of any sulphur source, and that on the fertilized plats the alfalfa contains a very much higher percentage of protein. Determinations were made in each sample of the total and nitrate nitrogen in order to ascertain whether there is any correlation between the sulphur and nitrogen content of alfalfa from the various sources.

It has been long established that sulphur is one of the ten essential elements for the growth of plants. This is easy to understand when one recognizes that sulphur is an essential building material for all plant proteins, as well as for various odor and flavor producing organic compounds found in members of the mustard family, onions, etc. While much of the sulphur in plants is found in the form of these organic compounds, there is also some inorganic or sulphate sulphur present. The latter is generally considered an excess of absorption of sulphur over its utilization. It may be, however, that some free sulphate sulphur must be present as a building material in order to insure maximum protein synthesis and maximum growth. SCHERTZ found that in the older leaves of *Coleus Blumei* some free nitrate must be present as a building

material in order to prevent the decomposition of organic nitrogen compounds—proteins, chlorophyll, phospholipines, etc. A similar situation may hold in alfalfa for sulphate as a sulphur source.

On the basis of WOLFF's old ash analyses of crop materials, agricultural scientists came to assume that so little sulphur was used by crops that there was no doubt that all soils furnished an abundance of this material. The magnitude of error likely to appear in the ashing method is well illustrated by the fact that WOLFF's analyses showed that one hundred bushels of corn contain 0.2 lb. of sulphur, while analyses of the modern fusion methods show at least 8.5 lb. or 42.5 times as much (2). Ashing seems to drive practically all of the organic sulphur off into the air, and determines only the small amount of sulphur existing in the inorganic form.

The use of the fusion method (7) of determining the sulphur content of crops has quite changed the situation by showing that all crops are considerable users of sulphur, and some crops very heavy consumers of sulphur. This fact, together with the general low percentage of sulphur in soils and the large losses of sulphur from soils by leaching, has shown that the question of sulphur supply to crops needs serious consideration. The Oregon (11) and Washington (9, 14) stations have shown beyond doubt that alfalfa cannot be grown successfully on many lands of those states without the addition of a sulphur source. They commonly get increased tonnage amounting from 100 to 500 per cent by the use of gypsum or other sulphur sources. The protein content of the hay is also increased almost 2 per cent in some cases by the use of sulphur fertilizers. From these facts it seems probable that the marked benefits received from the use of land plaster on legumes and other high sulphur-using crops in eastern United States and England during the last 150 years are due to gypsum furnishing an excellent sulphur source (3).

HART and PETERSON (5) of Wisconsin, BROWN and KELLOGG (2) of Iowa, and SHEDD (12) of Kentucky have all emphasized the fact that a permanent fertility system must look after the sulphur supply of the soil as well as the so-called three fertilizer elements—nitrogen, potash, and phosphorus. Recent work is indicating that

the same is true of calcium, and some suggest that the same may sometimes be true for magnesium. It is not within the scope of this paper to give a full discussion of the present status of the sulphur fertilization problems in the United States. For a critical discussion of this problem the reader is referred to a paper recently written by CROCKER (3).

Methods

In order to study the chemical composition of hay produced in various localities, samples were secured from three different states, Kansas, Illinois, and Missouri. So far as it was possible, these samples were taken from several different places in the mow. This may not have constituted so good a representative sample as could have been secured from the field, but it was the best that could be obtained under the circumstances. The samples were dried for some time in the laboratory and then finely ground and the moisture determined. The samples aggregated about 100 gm. of the finely ground material. Aliquots of these samples were used for all the determinations. Table I gives a list of the samples, and, so far as possible, the types of soils and locality from which

TABLE I

Sample	Locality	Soil type	Crop
1.....	Circleville, Missouri	Alfalfa
2.....	Meadville, Missouri	Grundy silt loam	Alfalfa
3.....	Brookfield, Missouri	Shelby loam	Sweet clover
4.....	Horton, Kansas	Silt loam	Alfalfa
5.....	Horton, Kansas	Brown silt loam	Alfalfa
6.....	Emporia, Kansas	Silt loam	Alfalfa
7.....	Emporia, Kansas	Silt loam	Alfalfa
8.....	Pratt, Kansas	Alfalfa
9.....	Paris, Illinois	Brown silt loam	Alfalfa

they were taken. The following facts are necessary for a complete understanding of the table. The samples taken from Missouri were from farms that had never been under alfalfa cultivation until the last two years. The sweet clover had been harvested but once and was purely an experimental crop. The samples taken from Kansas were from some of the oldest and best alfalfa fields in the state. Some of these fields had produced maximum

crops for the past twelve years. Particular attention must be given to the samples from Paris, Illinois. Part of this field had been an old orchard where alfalfa had never been grown before. When the hay was ready to be harvested, parts of the field showed a difference in color, some being yellow and others being dark green. These samples when analyzed showed a marked chemical difference, as shown in the tables.

Several methods of sulphur determinations were tried before satisfactory results warranted the adoption of any particular one. The peroxide method as described in the *Agricultural Chemists Bulletin* no. 107 was finally adopted. The Osborn method caused a great deal of trouble by the igniting or foaming over of the material near the completion of the first fusion. This may have been due to too rapid heating of the crucible. This difficulty was better controlled in the official method because of the presence of sodium carbonate, which slowed down the reaction. The carbonate also caused some trouble at first in removing the residue from the crucible. This, however, was overcome by allowing a small stream of water to play on the residue after it had cooled just enough to prevent spattering. When the crucible was filled with water the material came out very easily. This treatment ruined the crucible in a very short time, but it shortened the process considerably.

There were two distinct crucial periods in the process. The first was when the sodium peroxide began to break down the material and ammonia was being liberated. This was the most critical because of the flashing which caused the loss of many determinations. Samples varied considerably in the flashing, perhaps owing to the different amounts of nitrate present. The second critical point occurred in the slow heating after the addition of the first 10 gm. of peroxide. The flame had to be regulated and the stirring so constant that the reaction did not become violent enough to burn up the sample or cause it to foam over the top. When these conditions were carefully controlled, the solutions were clear when neutralized, and the results obtained from a series of duplicate samples agreed as well as could be expected.

The dry fusion method is severely criticized by KOCH (STOCKHOLM 13) because not all the sulphur is secured by this method.

He states that in any dry fusion method there is some sulphur being lost in the fumes no matter how carefully the fusion is made. No experiments were made to test this statement, but the variation in results obtained from a number of analyses of the same sample leads one to believe that even with the official method some of the sulphur is not secured. It seems probable that in all determinations of the sulphur content of plant materials to date, the results are low because of the loss of sulphur in fusion. Some difficulty was experienced in controlling the fusion so as to prevent the reaction proceeding too rapidly. If the reaction becomes too violent, smoke is evolved in considerable quantities, and results obtained under these conditions are uniformly higher than those obtained when the fusion takes place at a moderate rate. At first the samples were considered lost although no flashing took place, but later these samples were saved, and in every case showed a higher value than those that were controlled perfectly, and agreed very closely in the final result. The rate of fusion may have a great deal to do with the amount secured from the sample. This point will be studied more extensively later. The evidence is that all the sulphur was not being obtained from the samples. KOCH advocates the use of perhydro, a very concentrated hydrogen peroxide, in the determination of sulphur, but unfortunately this substance has not been on the market since the war, as Germany was the sole manufacturer. OLSON (8) used the Parr bomb, and, when certain precautions are followed, claimed for it advantages in speed and in assurance against losses of sulphur. Modification of the method is reported in connection with more recent determinations (9, 10).

All the determinations were made on the air dry material, and the percentage of sulphur calculated to the oven dry weight as follows:

Number of grams in aliquot	1.25	1.25
Weight of BaSO ₄ obtained	0.0328 gm.	0.0329 gm.
Percentage of sulphur in BaSO ₄	13.73
Percentage of moisture in sample	5.82	5.82
Percentage of sulphur in sample	0.382	0.382

The BENEDICT method (4) was tried, but proved very unsatisfactory because of the extreme tendency of the material to sputter

at the time of fusion. This method is highly recommended by its author to give very close checks, even though they are lower than those of the peroxide method. BENEDICT attributes these high results in the peroxide method to silica, which is entirely lacking in his method. The writer experienced no trouble with silica in using the official method, for at no time was strong alkali allowed to stand in contact with glass without being neutralized. The ease with which the material was removed facilitated quick neutralization. There was never a weighable amount of silica found in the solution.

Sulphate sulphur determinations

The method of determining sulphate sulphur was the same as that used by AMES (1). Five grams of the dry material mixed with one per cent of hydrochloric acid were shaken in the mechanical shaker for three hours. The solution was filtered and an aliquot of 2.5 gm. taken. This was then treated with barium chloride and precipitation allowed to take place in the cold. After standing for at least forty-eight hours the sulphates were determined in the usual way. The averages of the determinations are given in table II.

TABLE II

Sample no.	Moisture	Weight BaSO ₄	Total sulphur	Percentage total sulphur	Percentage sulphate sulphur	Total nitrogen	Nitrate nitrogen
Circleville, Missouri (5 gm.)							
1.....	7.58	0.1304	0.0179	0.387	0.01	2.41	0.01
2.....	7.59	0.1320	0.0183	0.397	0.01	2.42	0.01
3.....	0.1246	0.0182	0.371
4.....	0.1254	0.0171	0.373
5.....	0.1262	0.0173	0.375
Emporia, Kansas (1.25 gm.)							
1.....	5.81	0.0348	0.0047	0.405	0.013	2.32
2.....	5.82	0.0369	0.0050	0.424	0.012	2.31	Trace
3.....	0.0328	0.0045	0.382
4.....	0.0529	0.0045	0.383
5.....	0.0371	0.0051	0.432

TABLE II—Continued

Sample no.	Moisture	Weight BaSO ₄	Total sulphur	Percentage total sulphur	Percentage sulphate sulphur	Total nitrogen	Nitrate nitrogen
Brookfield, Missouri							
1.....	8.57	0.0354	0.0048	0.425	0.024	2.09	0.01
2.....	8.43	0.0337	0.0045	0.390	0.027	1.97	0.01
3.....	0.0319	0.0044	0.383
Horton, Kansas							
1.....	7.35	0.0430	0.0059	0.508	0.038	2.95	0.015
2.....	7.27	0.0476	0.0065	0.563	0.044	2.85	0.013
3.....	0.0429	0.0059	0.507
Horton, Kansas							
1.....	8.45	0.0365	0.0051	0.440	0.02	2.66	0.01
2.....	8.54	0.0310	0.0041	0.374	0.027	2.71
3.....	0.0369	0.0051	0.440
Meadville, Missouri (1.25 gm.)							
1.....	8.81	0.0345	0.0047	0.408	0.02	2.39	Trace
2.....	8.83	0.0346	0.0048	0.409	0.024	2.36
Emporia, Kansas							
1.....	8.11	0.0345	0.0047	0.412	0.04	2.47	Trace
2.....	8.12	0.0346	0.0048	0.413	0.042	2.44
Pratt, Kansas							
1.....	5.82	0.0307	0.0042	0.358	0.012	2.38	Trace
2.....	5.88	0.0319	0.0044	0.370	0.011	2.36
3.....	0.0318	0.0044	0.370
4.....	0.0308	0.0042	0.358
Paris, Illinois (green alfalfa)							
1.....	7.08	0.0425	0.0058	0.503	2.70	Trace
2.....	7.12	0.0414	0.0057	0.490	2.86
3.....	0.0419	0.0058	0.495
Paris, Illinois (yellow alfalfa)							
1.....	5.81	0.0331	0.0045	0.386	2.37
2.....	5.94	0.0326	0.0045	0.380	2.54

Total nitrogen

Total nitrogen was determined by the ARNOLD-GUNNING method as modified to include nitrate nitrogen. This method is described by MATHEWS (6).

Nitrate nitrogen

The nitrate nitrogen was determined by the SCHLESING-WAGNER method as modified by KOCH. The method is described in detail by WOO (15) in his chemical study of *Amaranthus*. To test the accuracy of this method a five-tenths per cent solution of potassium nitrate was used. Theoretically, 2 cc. of this solution should give 2.22 cc. of gas calculated to standard conditions. The average result of several determinations was 2.15 cc., which was about 97 per cent of the calculated amount. The precaution necessary to insure the success of the process as described by WOO is that all the solutions must be entirely free from oxygen. The presence of oxygen tends to cut down the amount of gas absorbed, thus causing a low result.

In making the determinations, aliquots containing 5 gm. of the original samples were extracted with two 100 cc. of water for one-half hour each. This extract was then treated with lead acetate to precipitate the proteins which caused much trouble unless removed. The samples were made up to volume of 250 cc. and filtered, then 100 cc. of the clear solution representing 2 gm. of the dry material was concentrated on the steam bath to about 20 cc. and the NO gas determined. The following represents a determination as run in duplicate from a sample:

Aliquots in cc. (2 gm.).....	100	
Total volume of gas evolved.....	1.66	cc.
Volume of unabsorbed gas	0.91	cc.
Volume of absorbed NO gas.....	0.75	cc.
Barometric pressure 749.7 mm., temperature..	24.5	
Volume at standard conditions.....	0.68	cc.
Equivalent milligrams of KNO ₃	0.0076	
Equivalent milligrams of NO ₃	0.005	

Discussion

It is important to note the variation in the sulphur content of the hay from the widely separated districts. From the tabulated

results it will be seen that the amount of sulphur in the various hays analyzed runs from 7.4 to 11.16 lbs. per ton of hay. If an acre produces from 5 to 8 tons of hay annually, 37 to 90 lbs. of sulphur will be removed from each acre of soil each year. The maximum figures here are much higher than those of PETERSON, because of the very high sulphur content of the samples from Kansas. The analyses of the samples from Missouri and Illinois give results that agree more nearly with the results of PETERSON'S analyses of alfalfa from Wisconsin.

One other marked deviation from the results reported by PETERSON is seen in the low amount of sulphate sulphur obtained in these analyses. He found that the ratio of the organic to inorganic sulphur was practically unity. In table II the sulphate sulphur in no case exceeds 10 per cent of the total sulphur in the crop, and in the samples taken from Illinois there was no sulphate sulphur. On the average for all analyses the sulphate sulphur equals 4.35 per cent of the total sulphur. The following shows the percentage of the sulphur that existed in the inorganic form in the alfalfa collected from various regions:

	PER CENT
Paris, Illinois (1)	0.0
Paris, Illinois (2)	0.0
Circleville, Missouri	2.5
Emporia, Kansas (1)	3.0
Pratt, Kansas	3.2
Meadville, Missouri	5.3
Brookfield, Missouri (sweet clover)	6.2
Horton, Kansas	7.6
Emporia, Kansas (2)	<u>9.9</u>
Average	4.35

Summary

1. Alfalfa hay grown in various parts of the United States shows considerable difference in the percentage of total sulphur content, quite independent of sulphur fertilization.

2. In general, hay from fields with the heaviest crops contains the highest percentage of sulphur.

3. Good to excellent crops of alfalfa hay remove annually from thirty-seven to ninety pounds of sulphur per acre, an amount which would seem far in excess of the amount returned by rain.

4. In some samples all, and in every sample more than 90 per cent, of the total sulphur was in the organic form. There was none or little sulphur present in excess of the actual needs as building material.

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